STAR Protocols

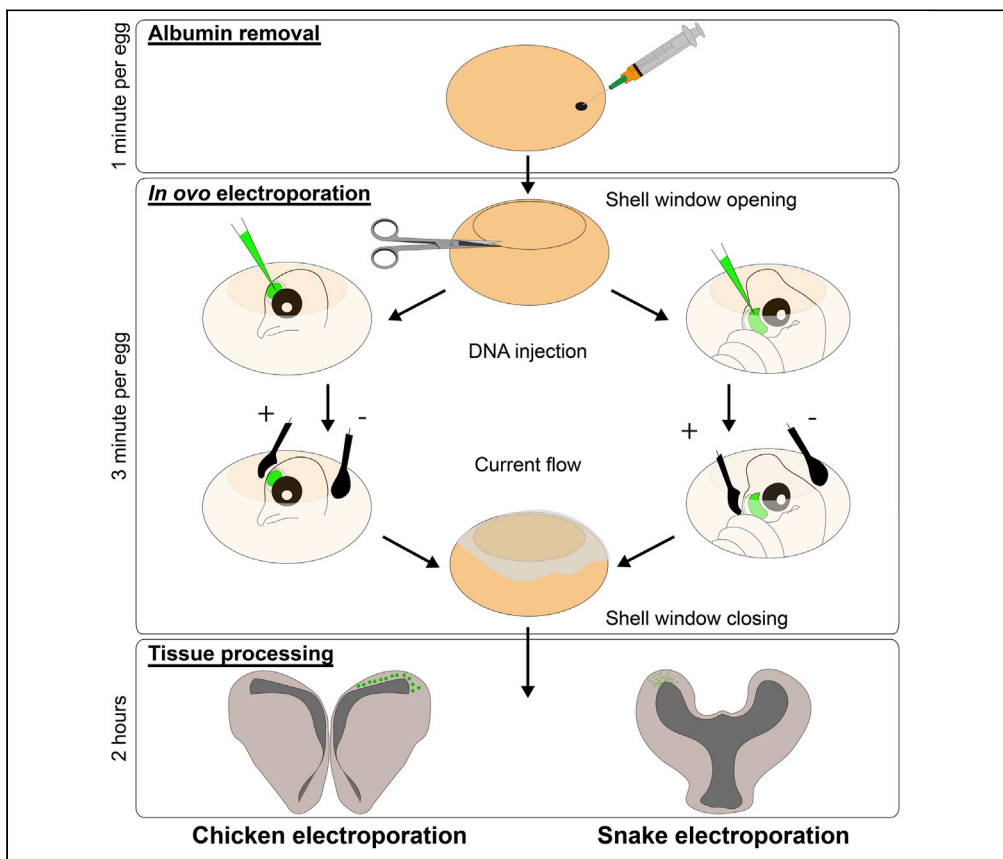**Protocol**

A protocol for *in ovo* electroporation of chicken and snake embryos to study forebrain development

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Highlights

In ovo electroporation of dorsal telencephalon in chick and snake embryos

Maximal viability of the embryos makes the protocol highly efficient

The simplicity of the procedure makes it accessible to non-expert researchers

Adaptable to any type of gene manipulation of the embryonic telencephalon

In vivo electroporation has become a key technique to study genetic mechanisms of brain development. However, electroporation of the embryonic pallium in oviparous species, interesting for evolutionary studies but distinct from *in utero* electroporation, is quite infrequent. Here, we detail the *in ovo* electroporation of the developing pallium in chick and snake embryos. This protocol allows gene manipulation through introducing exogenous DNA into brain progenitor cells and can be adapted to any type of gene manipulation of the embryonic telencephalon.

Protocol

A protocol for *in ovo* electroporation of chicken and snake embryos to study forebrain development

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SUMMARY

In vivo electroporation has become a key technique to study genetic mechanisms of brain development. However, electroporation of the embryonic pallium in oviparous species, interesting for evolutionary studies but distinct from *in utero* electroporation, is quite infrequent. Here, we detail the *in ovo* electroporation of the developing pallium in chick and snake embryos. This protocol allows gene manipulation through introducing exogenous DNA into brain progenitor cells and can be adapted to any type of gene manipulation of the embryonic telencephalon.

For complete information on the use and execution of this protocol, please refer to Cárdenas et al. (2018).

BEFORE YOU BEGIN

In vivo electroporation is a quick, simple and highly efficient method for introducing exogenous DNA into brain progenitor cells, with extremely accurate temporal and spatial control (Saito and Nakatsuji, 2001, Tabata and Nakajima, 2001, Borrell et al., 2005, Borrell, 2010). This is based on injecting the DNA or RNA of interest into the telencephalic ventricle of embryos, followed by passing pulses of electric current. Electric pulses produce small pores in the cytoplasmic membrane of progenitor cells that are in contact with the ventricular surface, mainly apical Radial Glia Cells, followed by the electrophoresis of DNA molecules, which are passed onto daughter cells upon subsequent mitotic divisions (dal Maschio et al., 2012).

In utero electroporation is commonly and widely used to manipulate the development of the cerebral cortex in embryos of small rodents, such as mice and rats, as well as of larger mammals like ferrets, with a special interest for studies of cortex folding (Kawasaki et al., 2012, Martinez-Martinez et al., 2016, Kalebic et al., 2018), with outstanding outcomes. Electroporation of embryos in oviparous species (*in ovo*) like chicken has also been used extensively to manipulate progenitor cells and study cell fate determination at early stages of development, classically in the spinal cord (Escalante et al., 2013, Saade et al., 2013). In contrast, electroporation of the developing cortex (pallium) in oviparous species like birds and reptiles is much more infrequent, despite its high potential for illuminating the genetic mechanisms underlying the expansion and complexification of the cerebral cortex during evolution (Nomura et al., 2013, Nomura et al., 2016, Garcia-Moreno and Molnar, 2015). Here, we describe a protocol to easily, economically and efficiently manipulate pallial progenitor cells in chicken and snake embryos by *in ovo* electroporation.

DNA preparation

⏱ Timing: 3 days

1. Prepare Luria-Bertani Broth (LB) medium + antibiotics. Make a 2% dilution of LB in sterile water and add the bacterial selection antibiotic at 1:1000 dilution from 50 mg/mL stock.
2. Prepare petri dishes for bacterial plating with 20 mL of 2% LB, 2% bacteriological agar and antibiotics diluted 1:1000 in sterile water. Keep at 4°C and pre-warm to 37°C before use.
3. Transform competent bacteria with the DNA of interest, plate the transformed bacteria onto petri dishes, and incubate at 37°C for 12–16 h. Transformation is performed by heat shock.
4. Individual bacterial colonies are clearly visible on the petri dish by the next morning. Remove the dish from the incubator and keep at 4°C until following to the next step.
5. Pick a single bacterial colony from the petri dish using a sterile yellow pipette tip, and transfer this to a 15 mL sterile tube with 5 mL of LB + selection antibiotics (1:1000 diluted). Incubate for 12–16 h, shaking at 170 rpm and 37°C. This pre-culture will be used as an inoculum to the MIDI-PREP culture.
   a. Prepare a MINI-PREP following the instructions provided in the kit. The resulting DNA will be used subsequently for sequencing.
6. Add the volume of the pre-culture into a sterile Erlenmeyer flask containing 200 mL of 2% LB + selection antibiotics (1:1000). Incubate overnight (14–16 h) shaking at 170 rpm and 37°C.
7. Purify the plasmid using the MACHEREY-NAGEL MIDI-PREP kit (Endotoxin free).
   a. The protocol included in the kit must be carefully followed.
8. Measure the concentration and purity of the resulting DNA suspension and store at −20°C for long term (years).

⚠ CRITICAL: All procedures must be done next to an open flame to maintain a sterile environment. All materials used must also be sterile.

PFA preparation

⏱ Timing: 30 min

9. For 100 mL of 4% paraformaldehyde in 1× phosphate buffer (PB), add 10 mL of solution A, 40 mL of solution B and 40 mL of distilled H_2O. Heat the solution to 60°C.

⚠ CRITICAL: Paraformaldehyde is highly toxic. Make sure to prepare it in a ventilated hood and using appropriate personal protective equipment, particularly nitrile gloves, safety goggles and lab coat.

10. Add 4 gr of paraformaldehyde powder to the hot solution and keep heating to maintain the temperature while mixing with a magnetic stirring bar and stirring plate.

Note: If the paraformaldehyde powder does not dissolve readily, slowly rise the pH by adding NaOH solution.

11. Once the paraformaldehyde is completely dissolved, adjust the pH to 7 if necessary with small amounts of HCl, and top-off to 100 mL with distilled water.
12. Filter and immediately cool on ice.

Note: The solution can be stored up to one month at 2°C – 8°C. However, it is highly recommended to make it fresh every time.

Egg preparation

Experimental procedures must be approved by the relevant Ethics Committee in each research institution. Fertilized chicken eggs are obtained from a poultry farm. The day of lay is considered day 0 post-ovoposition (dpo). Eggs are incubated in vertical position, with the larger pole on top, at 38.5°C in a hot air, rotating incubator. Fertilized snake eggs are obtained from a breeding colony. The day of lay is also considered 0dpo and eggs are incubated at 28°C and high humidity. Humid vermiculite is usually used as bedding for egg incubation, due to its capacity to retain both temperature and high humidity. It is important to maintain and manipulate the eggs always in horizontal position.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Chicken polyclonal anti-GFP	Aves Labs	Cat# GFP-1020, RRID:AB_10000240
Donkey Alexa488 anti-chicken IgY	Jackson ImmunoResearch	Cat# 703-545-155, RRID:AB_2340375
Bacterial and virus strains		
Escherichia coli DH5α	Thermo Fisher Scientific	Ref. 18265017
Chemicals, peptides, and recombinant proteins		
Fast Green FCF	Sigma-Aldrich	Ref. F7252
Agar (European Bacteriological Agar)	Conda Pronadisa	Ref.1800
LB Broth EZMix powder, dust-free, fast dissolving fermentation medium	Sigma-Aldrich	Ref. L7658
Ampicillin	Sigma-Aldrich	Ref. 10835242001
Kanamycin sulfate from *Streptomyces kanamyceticus*	Sigma-Aldrich	Ref. K1377
ClNa	Sigma-Aldrich	Ref. S9888
KCl	VWR	Ref. 26759.291
$Na_2HPO_4 \cdot 12H_2O$	Sigma-Aldrich	Ref. 71649
KH_2PO_4	VWR	Ref. 26925.295
NaH_2PO_4	Sigma-Aldrich	Ref. 71505
Na_2HPO_4	Sigma-Aldrich	Ref. S3264
Paraformaldehyde	Sigma-Aldrich	Ref. 441244
Sucrose	PanReac	Ref. 141621.1211
Frozen Section Medium NEG-50	Thermo Fisher Scientific	Ref. 6502
4,6-Diamidine-2-phenylindole dihydrochloride	Sigma-Aldrich	Ref. 10236276001
Critical commercial assays		
DNA, RNA, and protein purification	MACHEREY-NAGEL	Ref.740410.50
E.Z.N.A Plasmid DNA Mini Kit I	Omega	Ref. D6943-02
Experimental models: Organisms/strains		
Gallus gallus, fertilized chicken eggs	Granja Santa Isabel, Córdoba	www.granjasantaisabel.com; Cat#800008
Lamprophis fuliginosus, fertilized snake eggs	Laboratory of Michel C. Milinkovitch (scientific collaboration)	https://www.lanevol.org/
Recombinant DNA		
Expression plasmid: CAG-GFP	Addgene	N/A
Other		
Sterile tubes 13 mL	SARSTEDT	Ref. 62.515.028
Sterile petri dish	DB	Ref. 20443912002
50 mL Tubes	SARSTEDT	Ref. 62.547.254
10 mL Syringe	BD	Ref. 301156
21G × 1" Needle	BD	Ref. 307736
25G × 5/8" Needle	BD	Ref. 300600
Aspirator tube assemblies for calibrated microcapillary pipettes	Sigma-Aldrich	Ref. A5177-5EA
Borosilicate glass capillaries	World Precision Instruments, Inc.	Ref. 1B120F-4
Micropipette Puller	Sutter Instrument Co.	P-2000

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Electroporator	Intracel	TSS20 Ovodyne Electroporator
Current amplifier	Intracel	EP21
Electroporator	BTX	ECM 830
Tweezer electrodes	Nepa Gene	CUY650P3
Dissecting microscope	Leica	M60
Dissecting microscope	Zeiss	Stemi SV11
Dissecting scissors	FST	Ref. 14060-09
Spring scissors	FST	Ref. 91500-09
Forceps	FST	Ref. 91150-20
Minipulse 3 peristaltic pump	Gilson	Ref. SKU: F155001
Scotch adhesive tape	N/A	N/A
Wide adhesive tape	N/A	N/A
Sterile water	N/A	N/A
Piece of cork	N/A	N/A

MATERIALS AND EQUIPMENT

DNA dilution

Reagent	Final concentration	Amount
DNA solution (plasmid X)	Ranges from 10 ng/μL – 1 μg/μL	(depending on stock)
DNA solution (plasmid Y)	Ranges from 10 ng/μL – 1 μg/μL	(depending on stock)
Fast green 1%	0.1%	0.2 μL
Phosphate-Buffered Saline (PBS) 1×		Up to 20 μL
Total		20 μL

Micropipette puller setup

Puller parameters	Cycle parameters
Heat	650
Filament	3
Velocity	30
Delay	130
Pull	65

Electroporator setup

Electroporator parameters	Value
Pulse voltage	30 Volts
Programmable pulses	5
Pulse width	5 milliseconds
Space between pulses	500 milliseconds

10× PBS dilution

Reagent	Amount	Final concentration
NaCl	80 g	1.37M
KCl	2 g	27mM
$Na_2HPO_4 \cdot 12H_2O$	15.4 g	0.1M
KH_2PO_4	2 g	14 mM
Distilled H_2O	Up to 1 liter	

10× PBS stock dilution can be sterilized by autoclaving and stored indefinitely at room temperature, as long as sterility is maintained.

PB Solution A

Reagent	Amount	Final concentration
NaH$_2$PO$_4$·2H$_2$O	27.6 g	0.2M
Distilled H$_2$O	Up to 1 liter	

PB Solution B

Reagent	Amount	Final concentration
NaHPO$_4$	28.4 g	0.2M
Distilled H$_2$O	Up to 1 liter	

PB solution A and B can be stored at room temperature up to 3 months, if sterile conditions are maintained.

STEP-BY-STEP METHOD DETAILS
Albumin removal

⏱ Timing: 1 min per egg

This procedure allows moving the embryo inside the egg to an intermediate position in the horizontal plane, avoiding its damage when cutting a window on the shell, later on. This procedure is also described in the Methods video S1.

1. Clean the egg shell with 70% ethanol.
   a. For snake eggs, clean the shell and change the bedding from vermiculite to a double bottom setter tray. Add some clean warm water at the bottom to maintain the appropriate temperature.
2. Turn the eggs in the eggcup to a horizontal position and use scissors to make a small hole in the smaller pole of the egg. The size of the hole must be just sufficient to fit the thickness of the albumin extraction needle, around 1 mm.
   a. For snake eggs, skip this step.
3. Insert the 21G × 1'' needle fully through the hole in the shell off center, in a 45° angle directed towards the bottom part and one of the sides, to avoid extracting the chalaza o any membrane surrounding the embryo (Figure 1A).
   a. For snake eggs, use a 25G × 5/8'' needle.
4. Extract between 6–8 mL of albumin, depending on the size of the egg (Figure 1B).
   a. For snake eggs, remove 1–2 mL of albumin.
5. Use a piece of wide adhesive tape to cover the upper part of the egg and its hole. Be careful to avoid folding the tape (Figures 1C and 1D).

⚠ CRITICAL: Make sure to introduce the needle in the correct orientation to avoid damaging the embryo. The best time to remove the albumin is between lay and 3dpo, in both snakes and chicken. After this period, the viability of the embryo may be at risk during the process of albumin extraction due to the significant increase of the size and vascularization of embryos.

⚠ CRITICAL: The main limitation to manipulate the snake eggs is the softness of their shell. To solve this problem and avoid the collapse of the egg, surround it along the horizontal plane with narrow adhesive tape. Avoid covering the bottom part of the egg to allow the exchange of gas and humidity.

In ovo electroporation

⏱ Timing: 3 min per egg
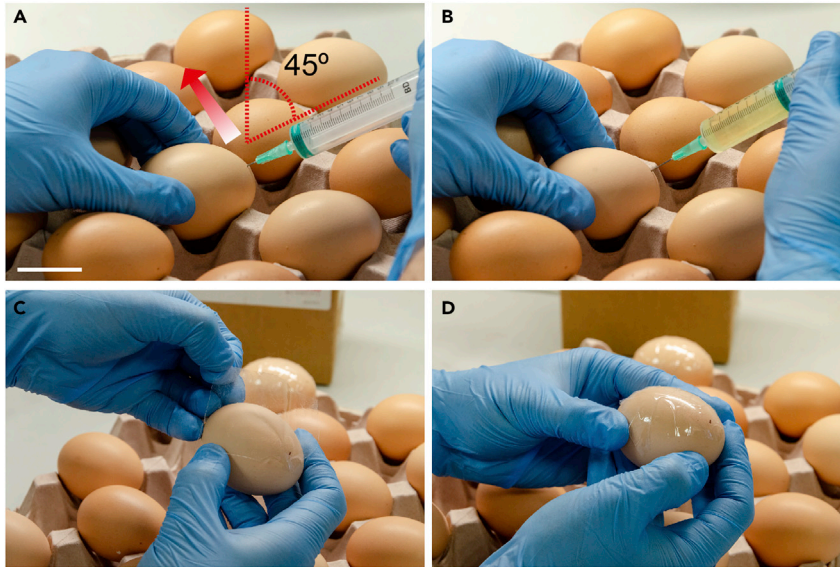
Figure 1. Albumin removal from chicken eggs
(A) Insert the needle at a 45° angle and direct it towards one side.
(B) Extract 6–8 mL of albumin.
(C and D) Cover the upper part of the egg with adhesive tape. Scale bar, 3 cm. See also Methods video S1.

This procedure allows injecting the DNA solution in the lateral ventricle of the chicken/snake telencephalon and introducing this DNA into progenitor cells that contact the apical surface of this ventricle, mainly apical Radial Glia Cells. Details of how key steps of this procedure are performed are shown in the Methods video S1. The best stage to perform the procedure in both animal models is 4 dpo, due to the positioning of the embryos in relation to the yolk. In addition, this is a key time point in the development of the dorsal pallium, since this stage is at the onset of neurogenesis. Therefore, electroporation at this stage will transfect mainly Radial Glia Cells that will later give rise to neurons.

6. Dilute the DNA plasmids of interest to the desired final concentration in PBS. As described in "materials and equipment", the final concentration of plasmids may vary across a wide range of concentrations depending on the specific experiment. The most common concentration used in our experiments is 1 μg/μL for each plasmid.

7. Clean all surgical materials, electrodes and surfaces with 70% ethanol.

8. Enter the electroporation parameters in the electric pulse generator and connect the tweezers electrodes.

    Note: For chick embryo electroporation we use TSS20 Ovodyne Electroporator, and for snake embryos we use ECM 830. The same current parameters should be used in both species. Both are square-pulse electroporators, so in principle both are useful for either species. Our different use was solely circumstantial related to availability.

9. Break the tip of the glass pipette in a beveled shape. The diameter of the tip should be around 50–100 μm.

    Note: This is important to facilitate penetration of the pipette into the ventricle of the embryo, causing the least possible damage.

10. Load the DNA solution into the glass pipette through the broken tip, using the aspiration tube. The aspiration tube has a silicone holder to hold the glass pipette. Mouth suctioning from the
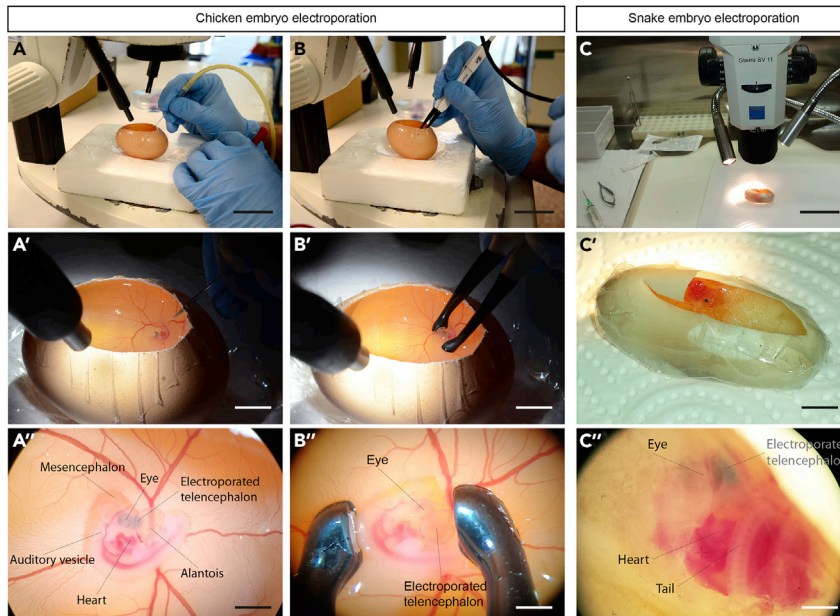
Figure 2. Electroporation process

(A, A') Injection of DNA dilution in the telencephalic vesicle of the chicken embryo. (A'') Detailed view of the injected telencephalon in chicken embryo.

(B, B') Position of the tweezers electrodes to allow the current flow. (B'') Detailed view of the position of the electrodes to electroporate the telencephalon.

(C) Set-up for snake embryo electroporation, (C') example of a window opened in the snake egg to manipulate the embryo, and (C'') detailed view of the electroporated snake telencephalon. Scale bars, 5 cm (A and B), 10 cm (C), 1 cm (A', B' and C'), 3 mm (A'' and B''), 2,5 mm (C''). See also Methods video S1.

other end of the tube creates a vacuum that will aspirate the DNA solution and load it into the pipette. Monitor the DNA loading to stop at the volume of interest. Troubleshooting 1.

11. Place the egg in the handmade holder on a horizontal position and use the scissors to open a window in the upper part of the shell. Start from the small hole made previously to remove the albumin, and follow along the adhesive tape.

Note: The size of the window should be the minimum to properly manipulate the embryo in order to avoid potential dehydration problems. In chicken eggs, windows with 1.5 – 2 cm of diameter should be sufficient, whereas in snake eggs these should be smaller, around 1 cm.

Note: Cut the shell with care, using the tip of the dissection scissor to avoid damage in the embryo or in the membranes surrounding it. Use the small spring scissors to cut the soft shell of the snake eggs. Troubleshooting 2.

Note: It is possible to make a holder with any material that allows performing a hole in the center to place the egg in a correct position and to avoid movements of the egg. In our case, we use a piece of polystyrene with a hole fitting the size of the egg, as shown in Figure 2.

⚠ CRITICAL: For snake embryos, the window on the egg shell must be made with extreme care, because the egg is smaller in size and the volume of albumin removed previously is also smaller, as the embryo is larger relative to the size of the egg.

12. Once the embryo is exposed through the window, put the egg under the dissecting scope to visualize and identify properly the telencephalon.

Note: The telencephalon is recognizable as a small bulged structure in the rostral part of the head, between the eyes. If the embryo is not positioned in the center of the window, move it carefully to that position, using the scissors if necessary. Approach the embryo with the scissors on horizontal position, let the embryo stick to them, and move both with extreme care.

13. Connect the pulled glass micropipette loaded with DNA solution to one end of the aspirator tube, introduce the pipette in one of the lateral telencephalic ventricles, and inject 1–2 μL of DNA solution by mouth blowing through the aspirator tube (Figures 2A–2A''). Troubleshooting 3.

    Note: The pipette must be introduced directed towards the part of the telencephalon to be electroporated. To electroporate pallial structures it must be introduced from the bottom of the telencephalon, and from the upper part to electroporate subpallial regions. Avoid injecting perpendicular to the telencephalic middle, because the DNA solution is frequently lost through the third ventricle.

    ⚠ CRITICAL: Introducing the pipette into the telencephalic vesicle must be done in a single and gentle push. Do not apply excessive pressure nor perform repeated attempts on the same embryo, as this will cause excessive damage on it. Troubleshooting 4.

14. Place the electrodes over the telencephalic vesicles in the position of interest, and press the switch for electric pulse discharge (Figures 2B–2B'').

    Note: DNA molecules will move towards the positive electric pole, so the precise placement and orientation of the electrodes critically determine the final result. The albumin is a good conductor of electricity, allowing a proper electroporation, so it is not strictly necessary to add an ionic solution to facilitate electrical conductivity.

    Note: To electroporate the dorsal pallium, the positive electrode must be located in parallel to the injected telencephalic vesicles and in close contact with the embryo. The negative electrode must be located in the posterior part of the embryo in a region between the heart primordium and the mesencephalon (Figure 2B'').

    Note: The electrodes look big next to the embryos, but this size does not affect their survival. It is possible to use smaller, needle electrodes, similar to those classically used to electroporate spinal cord, and this probably results in a smaller electroporated region. However, we have noted that the efficiency of the electroporation decreases significantly. It is also possible to target different regions just modifying the position of the electrodes.

    Note: The electrodes should not put pressure on the embryo, but only touch it gently. The rhythm of heart beating changes during electric pulses, and this should be used as an indication that electroporation is taking place. Pay attention that the heart beats again at a normal rhythm (similar to before electroporation) immediately after passing the electric pulses. Troubleshooting 5.

15. Cover the window on the shell with wide adhesive tape, avoiding air openings.

    Note: It is advisable to stick pieces of scotch tape surrounding the adhesive cover tape to help prevent any detachment.

16. Return the egg to the incubator in horizontal position and without movement.

Note: The addition of antibiotics into the egg is usually not necessary if the procedure was performed cleanly. It is also not necessary to cover the egg window with the piece of shell previously removed. The use of clear adhesive tape enables easy checking the viability of the embryo after the electroporation. Chicken eggs can be incubated under these conditions until the hatching day. For snake eggs, survival under these conditions is guaranteed until 8dpo (longer times not tested) (Figures 2C–2C'').

Tissue processing

⊙ Timing: 2 h

17. Prepare a Petri dish with ice-cold 1× PBS to keep the embryos after extraction from the egg. Sterile conditions are not necessary throughout tissue processing.
18. Prepare 50 mL tubes with ice-cold 4% PFA.
19. Cut out the adhesive tape covering the window in the shell and leave the embryo exposed.
20. While holding the embryo, carefully cut the membranes and the blood vessels surrounding it to allow its detachment.
21. Pull out the embryo with a small spoon or pour the content of the egg in a dish.
22. Small size embryos (less than 10 dpo):
    a. Extract each embryo and place in ice-cold PBS.
    b. Cut the head of the embryo and fix by immersion in cold 4% PFA during 60 min.
    c. Wash 2 times with cold PBS for one minute each in a 50 mL Falcon tube.
    d. Extract the brain by removing the skin and the incipient cranium under the dissecting scope by using sharp forceps (Figure 3). The procedure is the same for snake embryos.
    e. Transfer to 30% sucrose in PBS for cryoprotection. A good indicator to know that the brain is properly cryoprotected is that it sinks in sucrose solution. This process depends on the size of the brain and it could take between 12 and 18 h.
    f. Embed in tissue freezing medium, freeze and cut in the cryostat. The thickness of the section depends on the specific experiment, where 20 µm is standard for immunohistochemistry at these embryonic stages.

23. Big size embryos (equal or more than 10 dpo):
    a. Pour the content of the egg in a dish, extract the embryo and remove the membranes surrounding it.
    b. Fix by transcardiac perfusion: use a 25G × 5/8" needle to pass 20 mL of 4% PFA through the heart using a perfusion pump.

    Note: the perfusion flow will range from 8 to 12 ml/min depending on the size of the embryo.
    c. Cut the head and remove the skin and the cranium cutting through the midline. Pull either side of the cranium towards each side, exposing the brain, cut the base of the telencephalon and extract it. This procedure must be performed using sharp forceps.
    d. Post-fix the telencephalon with cold 4% PFA during 30–60 min.
    e. Wash 2 times with cold PBS for one minute each in a 50 mL Falcon tube.
    f. Cryoprotect the brain by submersion in 30% sucrose and cut at the cryotome.

Note: Cutting with cryotome, or vibratome without cryoprotection, is recommended over cryostat because of the large size of the telencephalon. In this case, the thickness of the sections is 40–50 µm.

EXPECTED OUTCOMES

This protocol allows the introduction of exogenous DNA into brain progenitor cells contacting the surface of the ventricle, useful to manipulate the expression of any gene of interest (Figure 4). In
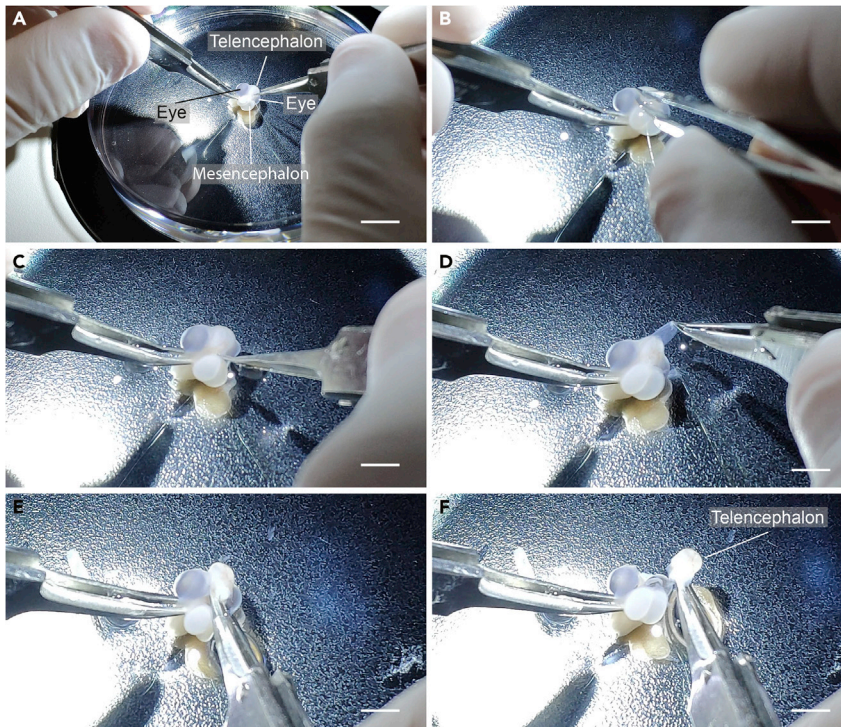
**Figure 3. Dissection process of the chicken embryo telencephalon at 6 dpo**
(A) Hold the head of the embryo puncturing the neck or the eye using one of the forceps.
(B) Using the other forceps, cut the skin laterally along one side of the telecephalon.
(C) Cut the skin between the telencephalon and the diencephalon.
(D) Remove the skin from the cut part.
(E) Cut the base of the telencephalon.
(F) Separate the telencephalon from the head. Scale bars, 1 cm (A), 600 μm (B – F).

addition, following this protocol it is possible combine the sequential injection of different reagents, such as DNA electroporation followed by Flash-Tag labeling or infection with retroviral vectors. The combination of these techniques allows custom-designed studies of early telencephalic development, including but not limited to progenitor cell behavior and neurogenesis.

This is an easy and optimized protocol to achieve an efficient electroporation with high rates of embryonic survival. In some cases, the electroporation of the egg is not possible because of the positioning or altered development of the embryo, such as for example a reduced size. For these reasons, only around 75–85% of eggs are suitable for electroporation. Under well-trained hands, the survival rate of electroporated embryos ranges between 90 and 95%, and if the protocol is performed correctly most of them will be successfully electroporated in the dorsal pallium (Figure 4C). In the case of snakes, the viability of embryos after electroporation is usually lower, down to 60–70%, due to their more complicated handling. The successful transfection of cells is also less efficient than in chick embryos, reduced down to approximately 50% of embryos (Figure 4D).

LIMITATIONS

The main limitation for the execution of this protocol is the developmental stage of the embryos. In both chicken and snake, starting at 4 dpo the head begins to sink into the yolk and the electroporation becomes extremely difficult, affecting the survival of the embryo and the success of the experiment. Secondly, the viability of electroporated embryos decreases slightly starting at the last third of the incubation period (14 dpo), but the survival of embryos remains relatively high, around 60% of the manipulated embryos.
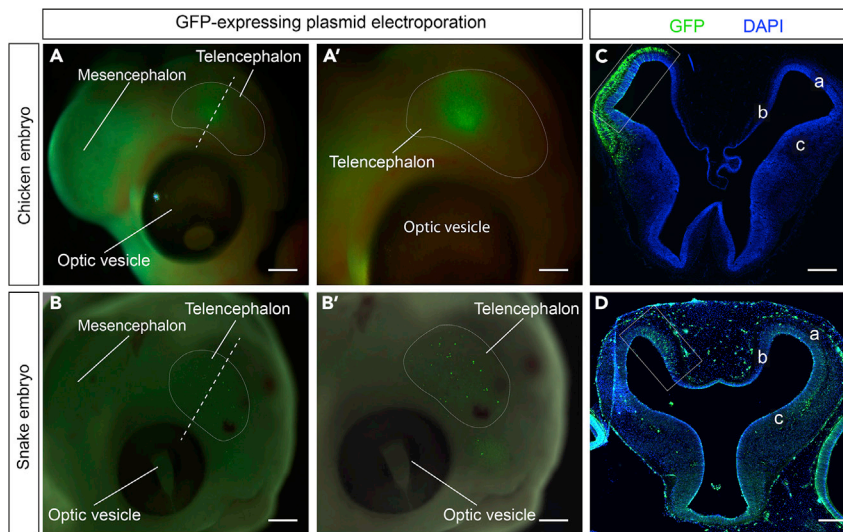
Figure 4. Expected outcomes

(A, A′) External view of the head of an electroporated chicken embryo.

(B, B′) External view of the head of an electroporated snake embryo. The limits of the telencephalon are indicated with a closed thin line.

(C and D) Coronal sections of the electroporated forebrain of chicken (C) and snake (D) embryos. The region containing GFP+ cells is highlighted with a white rectangle. (a) Hyperpallium in chicken and dorsal pallium in snake, (b) prospective hippocampus, (c) prospective DVR. Primary antibody used was: anti-GFP (1:1000, chicken polyclonal, Aves Lab). Secondary antibody used was: Alexa488 anti-chicken IgY (1:200, Jackson Immunoresearch). Scale bars, 400 μm (A and B), 300 μm (A′ and B′), 100 μm (C and D).

TROUBLESHOOTING

Problem 1

Problems loading the DNA solution into the glass pipette due to the viscosity of the DNA solution or obstruction of the tip (step 10).

Potential solution 1

The first potential solution to avoid this problem is to re-check the plasmid concentrations of your DNA solution, and that this is clean of solid particles or contamination.

If everything is correct and the problem persists, break again the very end of the pipette tip to facilitate the suctioning of the DNA solution, or to eliminate the obstruction.

The last option is to use a new pipette trying to break the tip with a bit thicker diameter while maintaining the sharpness.

Problem 2

Damage in the membranes or embryo during window opening (step 11).

Potential solution 2

Use only the tip of the dissection scissor to cut the shell of the egg and point this tip towards the upper part of the shell. If the problem persists, try removing an additional 1–2 mL of albumin in chicken eggs, or 0.5 mL in snakes.

Problem 3

Difficult accessibility to the telencephalic ventricles (step 13).

Potential solution 3

DNA injection is easier when the head of the embryo is positioned completely horizontal over the surface of the yolk. If this is not the case, gently rotate the embryo's head using the pipette, and then introduce it immediately into the ventricle.

Problem 4

Difficulty puncturing the telencephalon (step 13).

Potential solution 4

The glass pipette tip is likely not sharp enough to easily penetrate the tissue. Change the glass pipette and make sure you break it with a thin beveled tip.

Problem 5

Scarcity or absence of electroporated embryos (step 14).

Potential solution 5

This may occur due to a variety of reasons:

Low amount of DNA injected into the ventricle:

Check the DNA plasmid concentration, in stock and in the solution for electroporation.

Check the volume of DNA solution injected in each embryo. 1–2 μL of DNA solution should be sufficient to get a good electroporation. An easy way to measure this volume is to know how much was ejected from the pipette. Use a Hamilton syringe to make single-microliter marks in the glass pipette, as shown in the Methods video S1. If high precision is needed, the use of microprocessor-controlled injectors is recommended.

Make sure that the DNA solution is visible inside the lateral ventricle and it does not move away through the third ventricle.

Sometimes the pressure of the cerebrospinal fluid is high and pushes the DNA solution back out of the brain through the hole made with the pipette. Retract the pipette very slow and carefully.

Insufficient current flow:

Check that the parameters entered in the electroporator controller are correct, and check the cord connections.

Check that both electrodes make good contact with the tissue while passing the electric pulses. In the case of the pallium, place the negative pole touching the nape of the embryo and the positive pole in contact with the telencephalic vesicle while exerting a very gentle pressure.

After several embryos electroporated, albumin coagulates on the surface of the electrodes, making an insulating coat. Clean them appropriately with a paper humidified with distilled water to ensure appropriate current flow.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to, and will be fulfilled by, the lead contact, Víctor Borrell vborrell@umh.es.

Materials availability

No new plasmids, model animal lines or unique reagents were generated in this study.

Data and code availability

No new data or code was generated in this study.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100692.

AUTHOR CONTRIBUTIONS

Conceptualization, A.C. and V.B.; methodology, A.C.; writing - original draft, A.C; writing - review & editing, V.B. and A.C.; funding acquisition, A.C. and V.B.; supervision, V.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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